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Seasonal Assessment of Biomass and Fatty Acid Productivity by *Tetraselmis* sp. in the Ocean Using Semi-Permeable Membrane Photobioreactors S

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A green microalga, *Tetraselmis* sp., was cultivated in the coastal seawater of Young-Heung Island using semi-permeable membrane photobioreactors (SPM-PBRs) in different seasons. The microalgae in the SPM-PBRs were able to grow on nutrients diffused into the PBRs from the surrounding seawater through SPMs. The biomass productivity varied depending on the ion permeabilities of the SPMs and environmental conditions, whereas the quality and quantity of fatty acids were constant. The temperature of seawater had a greater influence than solar radiation did on productivity of *Tetraselmis* sp. in SPM-PBRs. SPM-PBRs could provide technologies for concurrent algal biomass and fatty acids production, and eutrophication reduction in the ocean.

Keywords: Marine photobioreactor, microalgae, ocean cultivation, semi-permeable membrane, *Tetraselmis*

Microalgae are considered as promising feedstocks for biodiesel production owing to their higher growth rate and oil productivity than other producers [1, 16]. However, the conventional algal culture systems generally require intensive power for temperature control, supply of nutrients, culture mixing, *etc.* [10, 11, 19, 26, 30]. Thus, microalgal culture strategies combined with treatment of wastewaters and utilization of flue gas have been recognized as cost-effective and eco-friendly strategies [2, 9, 12, 14, 29].

In these regards, the ocean could also serve as a place for algal cultivation with possible benefits from its large area, relatively constant temperature, and restless waves [3]. Additionally, seawater can be used for algal culture instead of freshwater [24, 27]. In particular, the West Sea of Korea has been suffering from eutrophication due to decades of ocean dumping. From another point of view, the West Sea is one of the most fertile regions in Korea. The feasibility of semi-permeable membrane photobioreactors (SPM-PBRs), allowing microalgae to grow on nutrients diffused into the PBRs from the surrounding seawater, for production of algal biomass and fatty acids (FAs) was demonstrated in a simulated ocean condition [15]. The productivities were directly correlated with the permeabilities of the SPMs. Although SPM-PBRs exhibited their feasibility for culturing microalgae, the results were obtained in controlled conditions. Since the environmental conditions continuously change in the ocean, the performances of the PBRs in the ocean and the effects of environmental factors on the productivities should be investigated.

In this study, *Tetraselmis* sp. was cultivated using SPM-PBRs with different permeabilities to evaluate algal biomass and FA productivities in seawater of Young-Heung Island, Incheon, Korea. We also analyzed the relationships between biomass productivities and environmental conditions.

A locally isolated green microalga, *Tetraselmis* sp. KCTC12236BP, was used owing to its high adaptabilities to the environment and to minimize the impact to the local environment in case of cell leakage. The cells were cultivated in artificial seawater supplemented with f/2-Si [15]. The seed culture was maintained in flasks at 18°C with continuous light of 50 μE m^{-2} s^{-1}. Biomass concentrations were measured using Multisizer 3 (Beckman Coulter, FL, USA). FAs were analyzed on a gas chromatograph (Younglin, Korea) [23]. Total inorganic carbon concentrations in seawater were analyzed by a TOC analyzer (Analytical Jena, Germany) [28]. NO_3^-, NH_4^+, and PO_4^{3-} concentrations in seawater were measured by an autoanalyzer (Bran+Luebbe, Germany). Temperatures were measured by temperature loggers (Shenzhen Everbest Machinery Industry, China). Data on solar radiation and sunshine duration at Young-Heung Island were obtained from the Korea Meteorological Administration (http://web.kma.go.kr/).

Experiments were conducted using PBRs made of four different semi-permeable membranes (PN. 132725, 132675, 132566, and 132544; Spectrum Laboratories, CA, USA), and the surface area of each SPM was 0.0816 m^2. Daily N and P transfer rates of the SPMs were ranked in the following order: A < B < C < D [15, 18]. SPM-PBRs, containing 30 ml of algal cultures, were wrapped in nylon nets and submerged at a 0.1 m depth. Each SPM-PBR culture was performed in three replicates (Supplemental Data 1). The nets were tied with ropes and anchored to a floating structure consisting of buoys in nearshore of Young-Heung Island (37.23°N, 126.43°E). Each ocean experiment lasted for 15–18 days in April, July, and November 2012, respectively.

The nutrients concentrations, pH, and salinity of the seawater are summarized in Supplemental Data 2. Significant seasonal variations of these parameters were not observed throughout the experimental periods. Considering the high dependence of algal growth in the SPM-PBRs on the nutrient transfer rates, the consistent seawater composition is favorable for reliable algal biomass and FA production.

The biomass productivities varied from 13.2 to 42.9 mg l^{-1} day^{-1} depending on the culture season and type of SPM (Fig. 1). The biomass productivity was directly proportional to the mass transfer rates of SPMs within a season. As the nutrients for algae were supplied from the surrounding seawater, microalgal growth was dependent on the nutrient permeability as shown in a previous study [15]. While the effect of nutrient permeability on biomass productivity of the SPM-PBRs was the same in all seasons, the deployment in summer resulted in the highest productivities.

The average daily solar radiation in spring (8.3 ± 1.8 MJ m^{-2} day^{-1}) and summer (10.7 ± 2.3 MJ m^{-2} day^{-1}) were higher than that in fall (5.0 ± 2.0 MJ m^{-2} day^{-1}). The atmospheric temperature fluctuated daily whereas seawater temperature remained constant during the cultivation period in each season (Fig. 2). Owing to the greater specific heat capacity of seawater than atmosphere, the seawater temperature did not increase or decrease quickly, resulting in approximately 5°C lower seawater temperature in spring than in fall. Generally, in outdoor cultures of microalgae, the major factor influencing biomass productivity is the light condition [8]. The conventional algal culture systems often aim to minimize the influences of factors other than light, such as temperature and nutrients, on biomass productivity [14, 20, 25]. Therefore, algal biomass productivity would be highest in summer, followed by spring and fall. In contrast, because it is difficult to control the seawater temperature, the culture temperature can be seen as a more critical parameter for biomass production than the light conditions for ocean cultures.

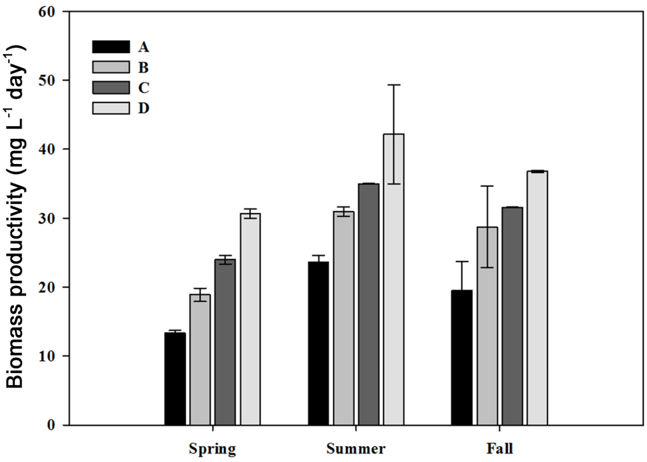

Fig. 1. Comparison of biomass productivities in SPM-PBRs with different nutrient permeabilities by seasonal deployments of SPM-PBRs in the coastal seawater of Young-Heung Island.
A, B, C, and D indicate the different types of SPMs.

In laboratory experiments, the optimal temperature range of *Tetraselmis* sp. culture was 18 ± 2°C. When cells were cultivated at 10°C, the biomass productivity decreased to 42.0 ± 3.5% of that at 15°C (data not shown). At a culture temperature above or below the optimal range, the algal growth rate could be sharply decreased [20]. Thus, despite the higher solar irradiance, the lower biomass productivity in spring than in fall was caused by the lower seawater temperature. Indeed, a linear relationship between seawater temperature and algal biomass productivity was found, whereas the solar irradiance was not correlated with algal biomass productivity (Fig. 3). One way to increase biomass productivity in spring would be culturing different

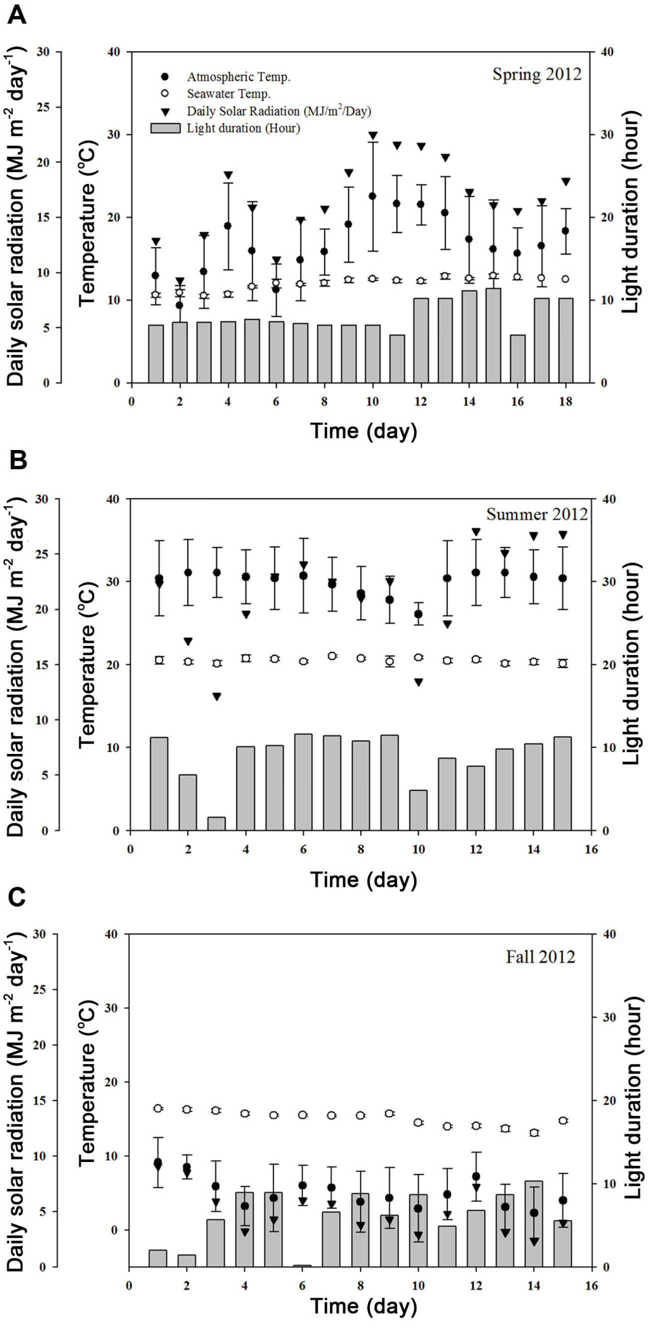

Fig. 2. Seasonal variations in atmospheric temperature (closed circle), seawater temperature (open circle), daily solar irradiation (inverse triangle), and duration of sunshine (gray bar) at Young-Heung Island during the *Tetraselmis* sp. cultivation periods.

(**A**) Spring 2012, (**B**) Summer 2012, and (**C**) Fall 2012.

microalgae in the colder seasons. Some microalgae have an optimal growth temperature as low as 4°C [22]. By culturing microalgae having an optimal growth temperature of

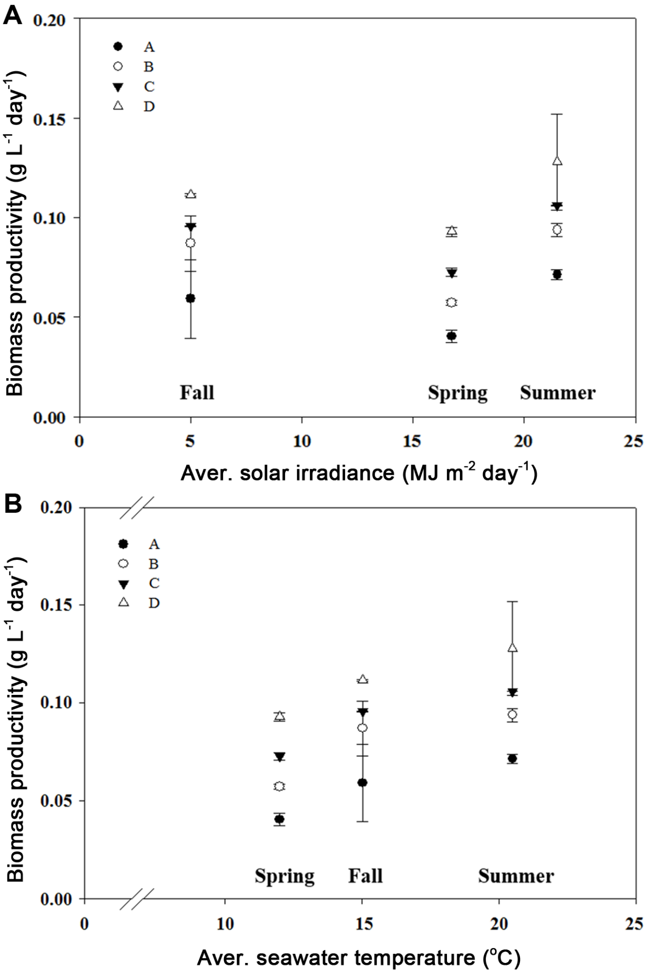

Fig. 3. Biomass productivities of SPM-PBRs as functions of average solar irradiance (**A**) and seawater temperature (**B**).

approximately 12°C, a significant increase in biomass productivity could be achieved in the spring.

Palmitic acid and oleic acid were the dominant FA accounting for 30.7 ± 2.3% and 27.1 ± 2.6%, respectively, followed by linolenic acid, hexadecatetraenoic acid, and linoleic acid (Table 1). There were no significant differences in FA compositions among the three seasons, contrary to other studies that reported low temperature promotes formation of unsaturated FAs [10, 25]. It has also been reported that the culture temperature could change the FA contents in some microalgae [4, 6], but significant changes in FA contents were not observed in this study. The FA productivities were increased in proportion to increased biomass productivities at higher temperature. The highest FA productivity obtained was 5.2 mg l^{-1} day^{-1} from SPM D in summer among all the SPM-PBRs in the three seasons. Compared with other culture systems, the productivity was

Table 1. Fatty acid composition, productivity, and content of *Tetraselmis* sp. seasonally cultured in Young-Heung Island using various SPM-PBRs.

	Spring				Summer				Fall			
	A	B	C	D	A	B	C	D	A	B	C	D
C16:0	32.5	33.5	30.5	34.5	31.2	33.2	30.9	28.5	27.9	28.3	28.7	29.1
C16:4	14.5	15.8	17.2	16.5	16.8	14.6	17.2	16.5	17.4	13.6	15.5	14.2
C18:1	30.2	30.5	27.5	29.5	28.3	26.8	29.1	24.2	25.2	22.2	26.8	24.9
C18:2	7.9	9.6	10.2	7.6	4.9	5.9	7.5	6.4	8.5	10.2	7.9	8.9
C18:3	14.9	10.6	14.6	11.9	18.8	19.5	15.3	24.4	21.0	25.7	21.1	22.9
ΣSFA[a]	32.5	33.5	30.5	34.5	31.2	33.2	30.9	28.5	27.9	28.3	28.7	29.1
ΣUFA[b]	67.5	66.5	69.5	65.5	68.8	66.8	69.1	71.5	72.1	71.7	71.3	70.9
FA productivity (mg l^{-1} day^{-1})	1.9	2.8	3.4	3.7	2.8	3.9	5.0	5.2	2.5	3.8	4.4	4.5
Total FA content (%)[c]	15.5	16.2	15.4	13.2	12.8	13.8	15.7	13.3	13.9	14.3	15.2	13.2

[a] Sum of saturated FAs.

[b] Sum of unsaturated FAs.

[c] Total FAs / DCW × 100.

similar to the values from raceway ponds (3–6 mg l^{-1} day^{-1}) [5, 17] and lower than those from closed outdoor PBRs (22–204 mg l^{-1} day^{-1}) [7, 21].

Microalgae were cultured using SPM-PBRs without supply of any nutrients or additional power consumption for algal cultures. Investigations on other aspects of marine PBRs, such as geometry, are also in progress. By applying the results to SPM-PBRs, better culture systems for the economic and sustainable production of microalgal FAs by utilizing ocean resources will be developed.

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References

1. Amaro HM, Macedo ÂC, Malcata FX. 2012. Microalgae: an alternative as sustainable source of biofuels? *Energy* **44:** 158-166.
2. Bhattacharjee M, Siemann E. 2015. Low algal diversity systems are a promising method for biodiesel production in wastewater fed open reactors. *Algae* **30:** 67-79.
3. Buckwalter P, Embaye T, Gormly S, Trent JD. 2013. Dewatering microalgae by forward osmosis. *Desalination* **312:** 19-22.
4. Converti A, Casazza AA, Ortiz EY, Perego P, Borghi MD. 2009. Effect of temperature and nitrogen concentration on the growth and lipid content of *Nannochloropsis oculata* and *Chlorella vulgaris* for biodiesel production. *Chem. Eng. Process* **48:** 1146-1151.
5. Crowe B, Attalah S, Agrawal S, Waller P, Ryan R, Van Wagenen J, *et al.* 2012. A comparison of *Nannochloropsis salina* growth performance in two outdoor pond designs: conventional raceways versus the ARID pond with superior temperature management. *Int. J. Chem. Eng.* **2012:** 9.
6. Durmaz Y, Donato M, Monteiro M, Gouveia L, Nunes M, Pereira TG, *et al.* 2009. Effect of temperature on α-tocopherol, fatty acid profile, and pigments of *Diacronema vlkianum* (Haptophyceae). *Aquac. Int.* **17:** 391-399.
7. Feng P, Deng Z, Hu Z, Fan L. 2011. Lipid accumulation and growth of *Chlorella zofingiensis* in flat plate photobioreactors outdoors. *Bioresour. Technol.* **102:** 10577-10584.
8. Grima EM, Fernández FGA, Camacho FG, Chisti Y. 1999. Photobioreactors: light regime, mass transfer, and scaleup. *J. Biotechnol.* **70:** 231-247.
9. Iancu P, Pleşu V, Velea S. 2012. Flue gas CO_2 capture by microalgae in photobioreactor: a sustainable technology. *Chem. Eng. Trans.* **29:** 799-804.
10. Jiang Y, Chen F. 2000. Effects of temperature and temperature shift on docosahexaenoic acid production by the marine microalgae *Crypthecodinium cohnii*. *J. Am. Oil Chem. Soc.* **77:** 613-617.
11. Jorquera O, Kiperstok A, Sales EA, Embiruçu M, Ghirardi ML. 2010. Comparative energy life-cycle analyses of microalgal biomass production in open ponds and photobioreactors. *Bioresour. Technol.* **101:** 1406-1413.

12. Kang Z, Kim BH, Ramanan R, Choi JE, Yang JW, Oh HM, *et al*. 2015. A cost analysis of microalgal biomass and biodiesel production in open raceways treating municipal wastewater and under optimum light wavelength. *J. Microbiol. Biotechnol.* **25:** 109-118.
13. Kim BH, Kang Z, Ramanan R, Choi JE, Cho DH, Oh HM, *et al*. 2014. Nutrient removal and biofuel production in high rate algal pond using real municipal wastewater. *J. Microbiol. Biotechnol.* **24:** 1123-1132.
14. Kim ZH, Lee HS, Lee CG. 2009. Red and blue photons can enhance the production of astaxanthin from *Haematococcus pluvialis*. *Algae* **24:** 121-127.
15. Kim ZH, Park H, Ryu YJ, Shin DW, Hong SJ, Tran HL, *et al*. 2015. Algal biomass and biodiesel production by utilizing the nutrients dissolved in seawater using semi-permeable membrane photobioreactors. *J. Appl. Phycol.* **27:** 1763-1773.
16. Lee SH, Ahn CY, Jo BH, Lee SA, Park JY, An KG, *et al*. 2013. Increased microalgae growth and nutrient removal using balanced N:P ratio in wastewater. *J. Microbiol. Biotechnol.* **23:** 92-98.
17. Lee SH, Oh HM, Jo BH, Lee SA, Shin SY, Kim HS, *et al*. 2014. Higher biomass productivity of microalgae in an attached growth system, using wast water. *J. Microbiol. Biotechnol.* **24:** 1566-1573.
18. Lee YK, Hing HK. 1989. Supplying CO_2 to photosynthetic algal cultures by diffusion through gas-permeable membranes. *Appl. Microbiol. Biotechnol.* **31:** 298-301.
19. Lin Q, Lin J. 2011. Effects of nitrogen source and concentration on biomass and oil production of a *Scenedesmus rubescens* like microalga. *Bioresour. Technol.* **102:** 1615-1621.
20. Ras M, Steyer JP, Bernard O. 2013. Temperature effect on microalgae: a crucial factor for outdoor production. *Rev. Environ. Sci. Biotechnol.* **12:** 153-163.
21. Rodolfi L, Zittelli GC, Bassi N, Padovani G, Biondi N, Bonini G, *et al*. 2009. Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. *Biotechnol. Bioeng.* **102:** 100-112.
22. Teoh ML, Chu WL, Marchant H, Phang SM. 2004. Influence of culture temperature on the growth, biochemical composition and fatty acid profiles of six Antarctic microalgae. *J. Appl. Phycol.* **16:** 421-430.
23. Tran HL, Kwon JS, Kim ZH, Oh Y, Lee CG. 2010. Statistical optimization of culture media for growth and lipid production of *Botryococcus braunii* LB572. *Biotechnol. Bioprocess Eng.* **15:** 277-284.
24. Trent J, Wiley P, Tozzi S, McKuin B, Reinsch S. 2012. Research spotlight: The future of biofuels: is it in the bag? *Biofuels* **3:** 521-524.
25. Wagenen JV, Miller TW, Hobbs S, Hook P, Crowe B, Huesemann M. 2012. Effects of light and temperature on fatty acid production in *Nannochloropsis salina*. *Energies* **5:** 731-740.
26. Wang J, Sommerfeld MR, Lu C, Hu Q. 2013. Combined effect of initial biomass density and nitrogen concentration on growth and astaxanthin production of *Haematococcus pluvialis* (Chlorophyta) in outdoor cultivation. *Algae* **28:** 193-202.
27. Yang J, Xu M, Zhang X, Hu Q, Sommerfeld M, Chen Y. 2011. Life-cycle analysis on biodiesel production from microalgae: water footprint and nutrients balance. *Bioresour. Technol.* **102:** 159-165.
28. Zeebe RE, Wolf-Gladrow D. 2001. *CO_2 in Seawater: Equilibrium, Kinetics, Isotopes*, pp. 4-11. Elsevier, Amsterdam, The Netherlands.
29. Zheng Y, Yuan C, Liu J, Hu G, Li F. 2014. Lipid production by a CO_2-tolerant green microalga, *Chlorella* sp. MRA-1. *J. Microbiol. Biotechnol.* **24:** 683-689.
30. Zhu LD, Hiltunen E, Antila E, Zhong JJ, Yuan ZH, Wang ZM. 2014. Microalgal biofuels: flexible bioenergies for sustainable development. *Renew. Sust. Energ. Rev.* **30:** 1035-1046.